## METHODOLOGY AND WORK PALN

### HPLC METHOD DEVELOPMENT

**Sample preparation**: It is a critical step during analysis. First consideration for developing the analytical methods is to determine the solubility of sample under analysis. Selecting specific solubility of bulk drug in a general solvent like water, methanol, Acetonitrile, Chloroform, hexane, ethanoletc. Mobile phase composition plays a key role in separation of different molecules. Mobile phase composition should be selected such way that it will not degrade the column packaging material and HPLC contact parts. Hence strong acids, bases and halide solution are avoided in mobile phase. Viscosities to be maintain low i.e. less than 0.5 centipoises in order to avoid the higher backpressure. The chemicals and solvents used should be high purity and easily available.

**Detector**: The selection of the detector is based on the chemical characteristic of the molecules under investigation. Due to the easy availability and as most of the chemical compounds shows absorbance in the UV Visible range, maximum method development starts from UV Visible detector. In some cases the molecules are converted into different form where the resulting compound shows absorbance in UV Visible range. When UV Visible response is not possible than only other types of detectors are considered.

**Columns** : Now a day's intensions is to develop an analytical method where the chromatograms should show maximum separation between different molecules within short run time and to use minimum solvent and protect the environment and cost , short length columns are preferred. The selection of Stationary phase to be selected based on molecule under analysis, generally reverse phases are more preferred e.g. C8, C18.In case of normal phases , most preferred stationary phases is Cyano (Nitrile phase.)

Subsequently the method is fine tuned selecting proper chromatographic Parameters like Flow rate, Column oven Temperature, Wave length, injection volume, tray temperature etc. Each HPLC analytical method should have few of the system suitability parameters such as Column efficiency, Resolution Factor, Tailing Factor, % RSD of peak area and % RSD of Retention times of multiple injections, Relative Retention Time, Similarity Factor, Capacity Factor, signal to noise ratio are complying to ensure the correctness of

analysis .These parameters are based on the outcome of the analytical method validation.

**Quality by design Approach (QBD)**: A systematic approaches to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. During the analytical method development as per QBD approach the variability occurring due to HPLC column, Mobile phase composition, pH etc are evaluated.

## ANALYTICAL METHOD VALIDATION

Analytical method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. FDA regulations such as GMP, GLP and GCP and quality standards such as ISO17025 require analytical methods to be validated before and during routine use.

The validation should follow a plan that includes the scope of the method, the method performance characteristics and acceptance limits.

All below parameters and evaluated during validation with predefined protocol and experimental conditions and validation results are documented in a validation report.

## SPECIFICITY

ICH defines specificity as "the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically this might include impurities, degradants, matrix, etc.

## PRECISION

ICH defines the precision of an analytical procedure as the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

## ACCURACY

ICH defines the accuracy of an analytical procedure as the closeness of agreement between the conventional true value or an accepted reference value and the value found.

Accuracy can also be described as the extent to which test results generated by the method and the true value agree.

## LINEARITY

ICH defines linearity of an analytical procedure as its ability (within a given range) to

obtain test results that are directly proportional to the concentration (amount) of analyte in the sample.

Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) or by separately weighing synthetic mixtures of the test product components.

Linearity is determined by a series of five to six injections of five or more standards whose concentrations span 80–120 percent of the expected concentration range. The response should be directly proportional to the concentrations of the analytes or proportional by means of a well-defined mathematical calculation.

**RANGE**

ICH defines the range of an analytical procedure as the interval from the upper to the lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

The range of an analytical method is the interval from the upper to the lower levels (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written.

**LIMIT OF DETECTION (LOD)**

ICH defines the detection limit of an individual analytical procedure as the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

**LIMIT OF QUANTITATION (LOQ)**

ICH defines the limit of quantitation (LOQ) of an individual analytical procedure as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

**RUGGEDNESS**

Ruggedness is not addressed in the ICH documents4.5. Its definition has been replaced by reproducibility, which has the same meaning. Ruggedness is defined by the USP as the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments,

**ROBUSTNESS**

ICH defines the robustness of an analytical procedure as a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. It provides an indication of the procedure's reliability during normal usage.

**SOLUTION STABILITY**

Solution stability is define storage period in solution form of standard solution by injecting the same standard solution after regular time interval.

**WORK PLAN:**

**Literature study.**

Literature Survey to find out the availability / non availability of High Performance Liquid Chromatographic methods, potential problems in available method Availability of material, Sample, isomer, Column, chemicals etc.

**Development and validation of analytical Method.**

Development of analytical method

Validation of developed analytical method and reporting of the results obtained.

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